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14. ABSTRACT
The goal of this proposal has been to determine whether a novel nanovector consisting of hydrophilic carbon clusters (HCCs) (pegylated) can serve as a therapeutic in a murine model of amyotrophic lateral sclerosis. HCCs were produced by Dr. James Tour of Rice University and provided to Dr. Grill to assess in his colony of G93A hSOD1 mutant mice. Aims were to determine whether PEG-HCCs functionalized with antibodies against the transferrin receptor could enhance lifespan, protect motoneurons and enhance motor function when delivered via sustained intravenous route at the first sign of disease. Second aim was to assess whether riluzole, in combination with functionalized PEG-HCCs could enhance the outcomes used in Aim 1. Progress in this grant was significantly reduced through two incidences with the Jackson Laboratory who had improperly sent us the wrong animals for the study. This resulted in a significant loss of time on two occasions as the colony needed to be refurbished. We report now, however that PEG-HCCs produce a significant enhancement in several indices of motor function, but not enhanced lifespain, when aapplied to the G93A mouse.

15. SUBJECT TERMS
ALS, inflammation, oxidative stress, motoneuron, nanovector, riluzole

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1. Introduction:

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that currently has no cure and results in a progressive neuropathology leading to death, usually within 3-5 years of diagnosis. Currently, riluzole is the only FDA-approved therapeutic for the treatment of ALS though it enhances lifespan by only a few months. There is a clear need for novel therapeutics that alone, or in combination with riluzole, can improve the lives of those living with ALS. Hydrophilic carbon clusters (HCCs) functionalized through the addition of polyethylene glycol subchains (PEG-HCCs) have recently been shown to: 1) exhibit potent antioxidant capabilities, and 2) possess the ability to serve as carriers for other potentially therapeutic molecules by James Tour, PhD of Rice University. The goal of this proposal was to determine whether functionalized PEG-HCCs could alone, or in combination with riluzole, enhance overall survival as well as preserve both motoneurons and behavioral function in mice that express a human mutated form of superoxide dismutase; a cause of ALS in a percentage of human familial cases of the disease. In this yearly report, we show that PEG-HCCs, under sustained intravenous delivery, produces a significant enhancement in several indices of motor function. We are currently in the process of finalizing motoneuron counts for this study and have requested until January 31st in a No Cost Extension request to complete this task.

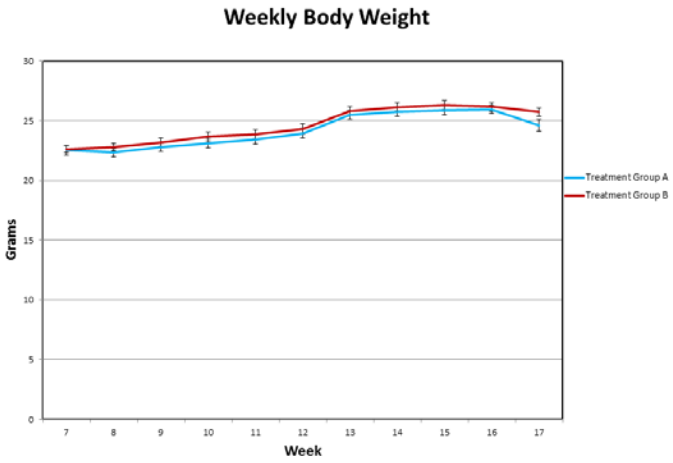
2. **Keywords:** ALS, nanovectors, riluzole, G93a, locomotor, vascular, antioxidant

3. Accomplishments:

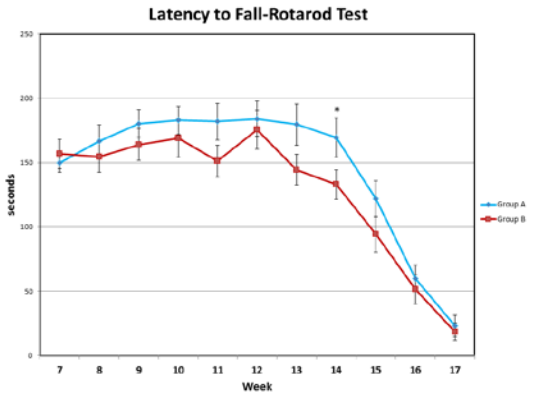
What were the major goals of the project: The goals of this proposal were: 1) to determine whether PEG-HCCs functionalized with antibodies against the transferrin receptor could enhance lifespan, protect motoneurons and enhance motor function when delivered via sustained intravenous route at the first sign of disease. Second aim was to assess whether riluzole, in combination with functionalized PEG-HCCs could enhance the outcomes used in Aim 1.

What was accomplished under these goals?
Due to problems encountered with Jackson Laboratories (described below) and the maintenance of our G93a mouse colony, our tasks for this time period were significantly reduced to assessing the therapeutic potential of PEG-HCCs in treating mice engineered to exhibit ALS-like symptoms. G93a mice were generated and maintained in our colony at UT-Health. Homozygous subjects were grafted with osmotic minipumps filled with either PEG-HCCs (condition A) created and provided by the Tour lab of Rice University or vehicle. These osmotic minipumps were implanted between the shoulder blades of the mice with a cannula leading from the minipump to the jugular vein. Thus, the PEG-HCCs or vehicle were allowed to provide a sustained delivery from the time where motor deficits were observed (around week 6) until subjects required euthanasia (based on inability to self-feed). During the treatment period, subjects were monitored for: 1) body weight, 2) balance via Rotorod assessment, 3) a variety of motor indices as determined via Photobeam Activity System assessement, and 4) lifespan. In a separate cohort of mice, lumbar anterior horn motoneurons were counted and compared between PEG-HCC and vehicle-treated subjects following euthanasia at day 110 (post-birth). Our results are as follows:

Body weight: We observe no difference in overall body weight throughout the treatment period between PEG-HCC- vs. vehicle treated subjects.

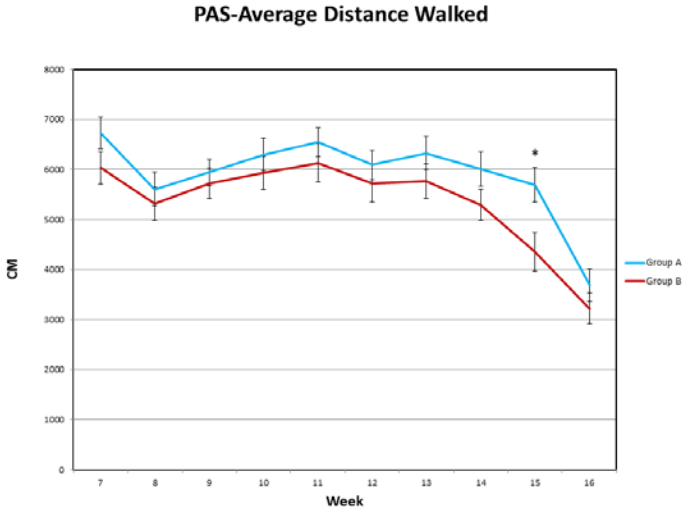


Rotorod (balance): G93a mice treated with PEG-HCCs showed a strong trend towards increased latency to fall on the rotorod test from week 7-15 with a statistically-significant enhancement detectable at week 14.



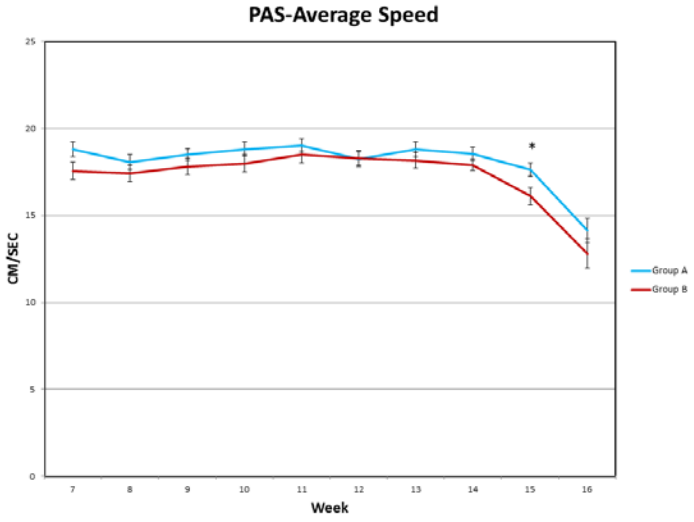
PAS test: Average Distance Walked

As with the rotorod test, PEG-HCC-treated mice showed a trend towards greater distances traveled compared to vehicle-treated subjects from weeks 7-16 with the enhancement becoming significant at week 15.

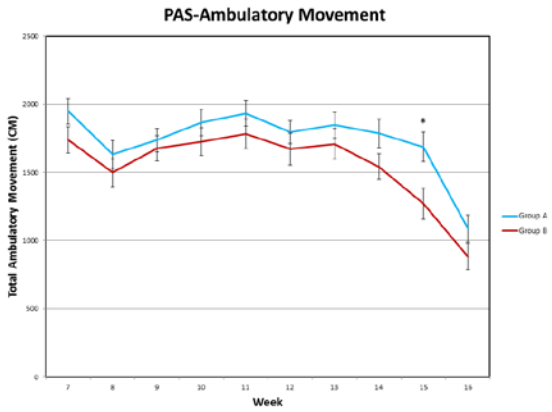


PAS test: Average speed

While placed in the PAS device, mice are allowed to ambulate freely for the entire period. The device measures the overall average speed of these movements. We observe a significant difference in speed favoring PEG-HCC-treated mice at week 15 compared with vehicle-treated.



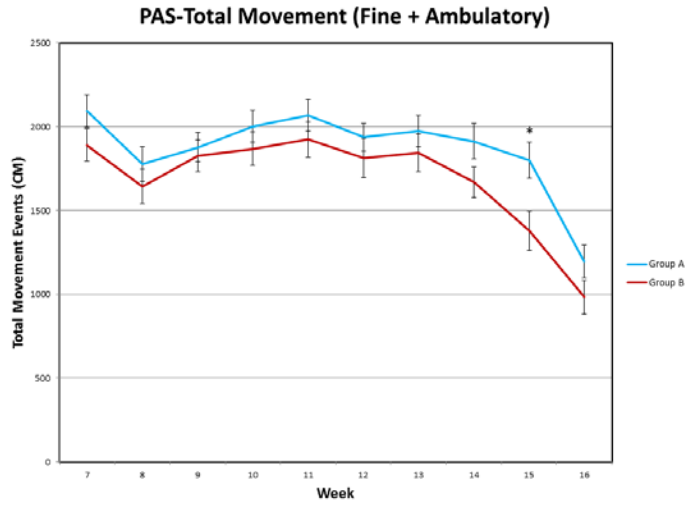
PAS-Ambulatory Movement



Ambulatory movement represents the total number of gross movements that the computer recognizes as longitudinal movement. Again, as with other previously described metrices, PEG-HCC-treated subjects exhibit a trend towards enhanced activity throughout the post-graft period while showing significantly enhanced ambulatory movement at week 15 post-grafting.

PAS-Total Movement (fine + ambulatory)

The PAS can also combine both fine and total ambulatory movements into one matrix. As you can see, PEG-HCC treated subjects again show a trend towards elevated movement with statistical significance achieved compared to vehicle-treated at week 15. Also similar to the other outcomes, both PEG-HCC and vehicle-treated subjects both undergo time-dependent loss of function regardless of therapeutic.



PEG-HCC vs. Vehicle-treatment: Preservation of spinal lumbar motoneurons

Due to the established antioxidant properties of PEG-HCCs, we hypothesized that sustained IV treatment would promote sparing of motoneurons at risk for degeneration in ALS as well as within the G93A mouse model. A separate cohort of mice were generated for the purpose of assessing motoneuron survival at day 110 in PEG-HCC vs vehicle-treated mice. Those motoneuron counts are based on immunohistochemical localization of a neuronal marker (choline acetyltransferase) and quantified image analysis. These studies are ongoing, but should be complete by the middle of January, 2015.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

As of yet, these data have not been introduced to the general research community. We are waiting on the results of the neuronal counts before deciding on a method of dissemination; though likely it will manifest as a short report/manuscript.

What do you plan to do during the next reporting period to accomplish these goals?
We have requested a No Cost Extension to conclude at the end of January, 2015 in order to complete the ongoing neuronal cell counts.

4. Impact

What was the impact on the development of the principle disciplines of the project?
Our observed results suggest that PEG-HCCs may have beneficial properties as a treatment for ALS that are worthy of ongoing development. PEG-HCCs were shown to produce significant effects on several behavioral matrices during the sustained treatment period compared to vehicle. Due to adverse issues associated with year 1 and the colony, were only able to assess PEG-HCC vs vehicle treatment. However, this comparison suggests a beneficial role of the bare PEG-HCC nanovector without further functionalization. It is our hope that we may continue developing functionalized PEG-HCCs with Dr.Tour's group in order to produce a novel and effective treatment for ALS.

What was the impact on other disciplines?
The potent anti-inflammatory properties of PEG-HCCs represent a novel potential therapeutic for spinal cord injury studies, another area of research interest in my laboratory.
What was the impact on technology transfer?
Nothing to report as of yet. We are awaiting to see what the results are on the neuronal counts prior to meeting with UT and Rice's Offices of Technology Transfer.

What was the impact on society beyond science and technology?
Nothing to report.

5. Changes/Problems

Changes in approach and reasons for change.
As described in a previous report, we encountered two problems with animal purchases through Jackson Laboratories. On two consecutive occasions, they provided us with the wrong animals which lead to our generating animals that lacked the human mutated SOD 1 gene. While Jackson Laboratories agreed to replace the animals originally purchased, we were unable to get them to recompense our loss of time and effort. As these studies require a significant amount of time to set up (purchase, breeding, behavioral assessments, etc), this resulted in a significant setback for our originally designed studies. Despite this, we have completed the behavioral assessment of PEG-HCCs vs. vehicle-filled osmotic minipumps. The results from these studies are encouraging and should lead to new funding applications through both federal and foundation sources. In addition, we are awaiting final outcomes on the neuronal survival counts prior to determining where to submit our report for publication.

Actual or anticipated problems or delays and actions or plans to resolve them
See immediately above.

Changes that had a significant impact on expenditures
We were left with finances of which we have requested a small amount to use towards personnel effort applied for the described ongoing cell counts.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
None to report.

Significant changes in use or care of human subjects
None to report.

Significant changes in use or care of vertebrate animals

none to report

Significant changes in use of biohazards and/or select agents
none to report

6. Products

publications, conference papers, and presentations
None to report. We do not wish to present our current data until we have finished analyzing the neuronal survival counts.

websites or other internet sites
None to report

Technologies or techniques
None to report until we know of the cell count outcome.

Inventions, patent applications and or licenses
Based on the final neuronal cell survival data, we will meet with the Tech Transfer groups at both UT-Health and Rice University prior to the end of January 2015.
Other Products:
None to report

7. Participants and Other Collaborating Organizations:

What individuals have worked on the project?
James Tour, PhD
Co-PI
0.25 Summer months
Dr. Tour developed and provided the PEG-HCCs as needed throughout the projet
Funding for this effort provided through this DOD-sponsored grant.

Has there been a change in the active other support of trhe PD/PI or senior/key personnel since the last reporting period?
None to report.

What other organizations were involved as partners?
None to report.

8. Special Reporting Requirements

Collaborative Awards
Dr. Tour's group has been responsible for providing Dr. Grill's group with the PEG-HCCs throughout year 1 and 2 of the project.

Quad Charts sent to CDMRP